主論文

岡本浩二

ENZYMATIC STUDIES ON THE FORMATION OF 5-KETOGLUCONIC ACID BY ACETOBACTER SUBOXYDANS

(I) Glucose Dehydrogenase

By KOJI OKAMOTO[§]

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Although the main pathway of carbohydrate metabolism in plants, animals and bacterial systems have been elucidated, biochemical fates of a certain derivatives of carbohydrates found in particular organisms have still remained obscure. One of these compounds is 5KGA[§§] which is accumulated in large amount in the culture medium of Acetobacter suboxydans. Under a proper condition of cultivation, glucose added in the medium was reported to be converted into 5KGA with 90% yield. However, details of the biosynthetic and degradative pathways of this keto acid is not known.

For the cultivation of this bacterium, $CaCO_3$ has to be added to neutralize acids produced by the fermentation. As the bacterium grows $CaCO_3$ dissolves into solution. Then after the nearly complete solubilization of $CaCO_3$, 5KGA crystallizes out. The original strong reducing power of the culture diminishes with time and reaches to the minimum when $CaCO_3$ dissolves completely,

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§§ Abbreviations: 5KGA, 5-ketogluconic acid; GA, gluconic acid; $NADPH_2$, reduced form of NADP.

and then increases to a considerable extent.

From these observations 5KGA has been supposed to be produced *via* two steps: glucose is first dehydrogenated to GA and subsequent oxidation of GA gives rise to this keto acid.

Although the first step of glucose oxidation by the particulate enzyme of *Acetobacter suboxydans* was studied by King *et al.* (1), little has been known about the glucose oxidation by the soluble enzyme of this microorganism except that a brief report by DeLey *et al.*(2) is available.

The present paper deals with the partial purification and characterization of the soluble glucose dehydrogenase of *Acetobacter suboxydans*. The results show that this enzyme is the new type of glucose dehydrogenase and is distinguished in many respects from both that of other sources and the particulate enzyme of this microorganism. The enzyme participating in the further oxidation of GA to 5KGA will be described in the subsequent paper (3).

E X P E R I M E N T A L

Microorganism *Acetobacter suboxydans* IFO 3432 was supplied from Institute for Fermentation Research and throughout these experiments. Prior to inoculation 1.5% of sterile dry $CaCO_3$ was added to the medium containing glucose (2%) and yeast extract (Oriental) (0.25%). Cultivation was carried out at 28°C for 30 hours on a shaking device. At the end of this period, excess of $CaCO_3$ was removed by low speed centrifugation (3000 r.p.m. x 5 minutes) and cells were collected, washed twice with cold saline.

Chemicals NAD and NADP were purchased from Sigma Chemical

Co. In some experiments, nucleotide mixture prepared from pig liver according to LePage et al. (4) was used in place of NADP. Mannoic acid was prepared by the oxidation of mannose with bromine water. Other chemicals were obtained from commercial sources.

Analytical Methods Reducing power was determined by Nelson's method (5). GA was determined by Lipman-Tuttle's method (6) after GA was converted into lactone by heating the sample (in 0.1 N HCl) in the boiling water for 3 minutes. Under the condition, absorbance at 450 mu was found to be proportional to the amount of GA between 0.5 and 3.0 μg. The amount of NADPH was estimated from the absorbance at 340 mμ by using the value $\varepsilon_{340} = 6.22 \times 10^6$ cm. per mole. Protein was determined by Lowry's (7) or Warburg-Christian's (8) method. Ascending paperchromatography of GA and mannoic acid was performed with two solvent systems. Rf values and the methods of color development were shown in the separate paper (3).

Assay for Enzyme Activity The activity of the enzyme was assayed through the measurement of the optical density increase (at 340 mμ) resulting from the reduction of NADP. The complete system contained 0.15 μmole of NADP, 10 μmoles of the substrate, 0.1 ml. of the enzyme in the total volume 2.8 ml. (Tris 0.1 M, pH 8.5). One unit of the enzyme is defined as the amount which will cause an initial (1 minute after the start of reaction) optical increase of 0.001 at 340 mμ.

R E S U L T

Enzymatic Reduction of Glucose

When cell extracts were incubated with NADP in the presence of glucose plus ATP, reduction of NADP was observed. Omission

of ATP from the reaction mixture was found to affect a little even with $(NH_4)_2SO_4$ treated enzyme. Any significant increase at 340 mμ was not observed when NAD was used in place of NADP. These observation suggested that TPN-linked dehydrogenation of glucose takes place without prior phosphorylation. Therefore partial purification and characterization of the enzyme was carried out.

Purification of the Enzyme

Soluble Fraction All the procedures were carried out at 0 - 5°C unless otherwise mentioned. One part of washed cells were ground with 2 parts of Alumina W-800 (Wako Pure Chemicals Co.) in a cold mortar and extracted with 10 times its weights of Tris buffer (0.01 M, pH 7.4). After removal of cell debris and alumina by two cycles of centrifugation (6000 r.p.m. 15 minutes), resulting supernatant (crude extract) was further centrifuged at 10,000 xg for 2 hours to remove fine particles. (soluble fraction)

1st. $(NH_4)_2SO_4$ Fractionation To 80 ml. of the soluble fraction 19.5 g. of solid $(NH_4)_2SO_4$ were added to give 0.40 saturation. After standing for 20 minutes, the precipitate was removed by centrifugation. Then 17.0 g. of $(NH_4)_2SO_4$ were added to the supernatant fraction to bring the concentration to 0.70 saturation. The precipitate was dissolved in 80 ml. of Tris-buffer.

Acrinol Treatment To 70 ml. of the above solution 7 ml. of acrinol solution (0.1%) were added drop by drop with constant stirring. After standing for 30 minutes yellow voluminous precipitate was centrifuged off. To the yellow supernatant (70 ml.) were added 7 ml. of 10% suspension of charcoal to remove excess acrinol. After standing for 10 minutes in the room temperature,

charcoal was removed by filtration. Resulting filtrate was then dialyzed against Tris-buffer overnight. Although the specific activity was not so raised by acrinol treatment, this step was found to render subsequent steps reproducible.

2nd. $(NH_4)_2SO_4$ Fractionation To 70 ml. of the dialyzate 22 g. of solid $(NH_4)_2SO_4$ were added to give 0.50 saturation. After the precipitate was removed by centrifugation, the supernatant fluid was brought to 0.70 saturation of $(NH_4)_2SO_4$ and the fraction obtained was dissolved in 40 ml. of Tris-buffer.

Acetone Fractionation Acetone, cooled to -10°C, was added slowly with stirring to achieve the final concentration of 38% by volume. The temperature of the solution was gradually allowed to drop to -20°C as acetone was added. The precipitate was centrifuged off and the supernatant was brought to 50% (V/V) of acetone. The resulting precipitate was dissolved in 25 ml. of Tris-buffer and used in the experiments described below. A typical example of the purification was summarized in Tab. I.

Table I

Purification of the Enzyme

	Volume (ml.)	Total activity (units)	Specific activity (units/mg.prot.)	Yield (%)
Soluble fraction	80	82,500	116	100
$(NH_4)_2SO_4$ fraction (40 - 70%)	80	59,700	250	72
Acrinol fraction	96	56,800	295	69
$(NH_4)_2SO_4$ fraction (50 - 70%)	30	33,100	540	40
Acetone fraction (35 - 50%)	20	27,400	2210	33

Properties of the Enzyme

Coenzyme Specificity As can be seen from Fig. 1, NADP was exclusively effective in this reaction. Since the same result was obtained with crude extract, this enzyme was thought to be responsible for the dehydrogenation of glucose in the soluble fraction.

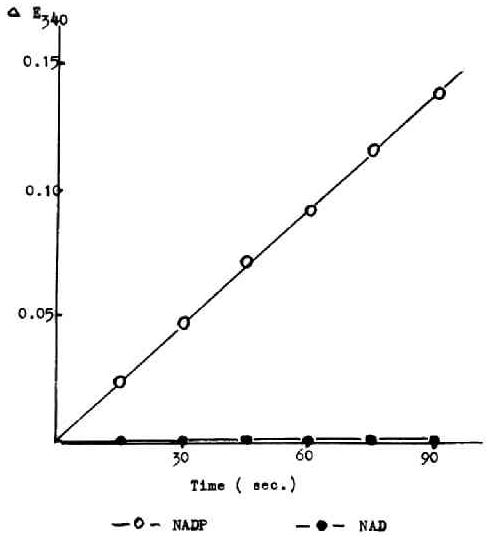

Fig 1. Coenzyme Specificity

Experimental conditions are described in the text except that NAD was used in place of NADP w here indicated.

Effect of pH The optimum pH for the reaction was found to be about 8.5 as shown in Fig. 2. The nature of the individual buffer used had little effect on the enzyme activity.

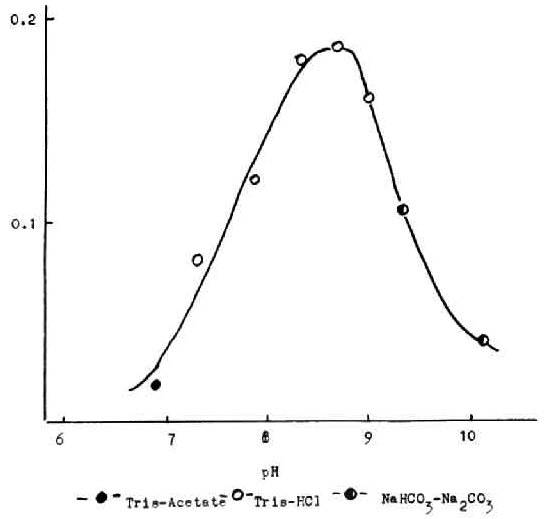

Fig. 2 Effect of pH

Experimental conditions are described in the text except that indicated buffers were used.

Substrate Specificity Of the several aldoses examined, only glucose and mannose were dehydrogenated by this enzyme. (Table II).

Table II

Substrate Specificity

Substrate	per cent activity
D-Glucose	100
D-Mannose	124
D-Galactose	2
D-Xylose	0
D-Lyxose	0
L-Arabinose	0
D-Ribose	0
D-Gluconic Acid (60 μmole)	0

Assay conditions : see the text.

The ratio of the activity of the enzyme towards glucose and mannose remained essentially constant throughout the purification procedure. Since GA was not dehydrogenated even in high concentration (20 μmoles per ml.) it may be thought that this preparation was free from the enzymes catalyzing further oxidation of GA.

Michaelis Constant Michaelis constants were determined for glucose and for mannose. The Km for glucose (5.3×10^{-3} M) was found to be greater than that for mannose (4.1×10^{-4} M) at the concentration of 5.4×10^{-4} M of NADP. (Fig. 3)

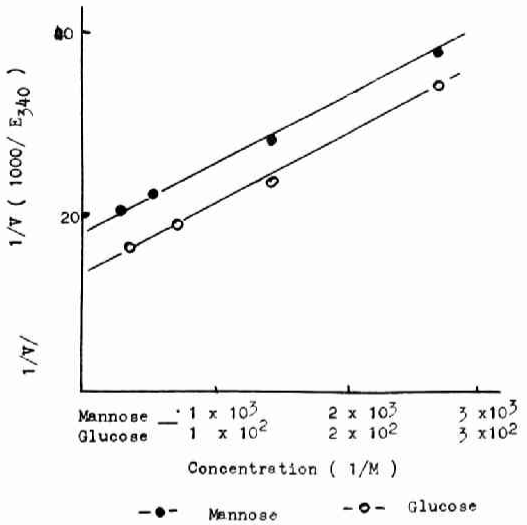

Fig.3 Relation between substrate concentration and activity
Assay conditions : See the text.

Reaction Product The products of the enzymatic dehydrogenation of glucose and mannose were identified by paper chromatography after relatively large scale incubation with the system in which TPN was regenerated. Incubation system contained 300 μmoles of glucose, 1μmole of TPN, 400 μmoles of α-ketoglutarate, 1 mole of $(NH_4)_2SO_4$, 9000 units of crystalline glutamic dehydrogenase§ from beef liver, 4 ml. of glucose dehydrogenase preparation, and 1 mmole of Tris in the final volume 10 ml. The solution was adjusted to pH 8.5 by adding 1 N H_2SO_4. After incubation for 3 hours at 30°C reaction was stopped by immersing the tube into the boiling water and the deprotenized solution (adjusted to pH 7.2) was applied to the Amberlite IR 120 column

§ Kindly supplied by Dr. Watari of Osaka University.

(H^+ form). The effluent was concentrated in vacuo after the removal of sulfate ion by treating with $Ba(OH)_2$. The concentrated product was then converted to the lactone by heating at 100°C for 3 minutes in the presence of 0.1 N HCl. Ascending paper-chromatography of the reaction product in two solvent systems revealed one distinct spot which was identical to that of the authentic gluconolactone.

The similar experiment was performed with mannose instead of glucose. GA and mannoic acid were clearly separated each other by paperchromatography in both solvent systems. (see EXPERIMENTAL) The result obtained showed that glucose and mannose were dehydrogenated by this enzyme preparation to the corresponding aldonic acid without prior interconversion.

Stoichiometry of the Reaction In the presence of an excess of TPN and relatively large amount of the enzyme, glucose was almost completely converted to GA, an equivalent quantity of TPN was reduced, with the concomitant formation of an equal amount of GA. (Tab. III)

Table III

Stoichiometry of the Reaction

Glucose	$NADPH_2$	GA
- 4.6 μmoles	+ 4.5 μmoles	+ 3.9 μmoles

Incubation: Glucose 5 μmoles, NADP 5 μmoles, enzyme 0.5 ml., total 3.0 ml. ($NaHCO_3$, pH 8.5) 30°C 5o minutes. For the determination of glucose, $NADPH_2$ and GA. see the text.

This balance is in accord with the equation:

$$\text{Glucose} + \text{NADP} + H_2O \longrightarrow \text{GA} + \text{NADPH} + H^+$$

Reversibility and Primary Product Although TPNH was not appreciably oxidized by this enzyme preparation even in the presence of GA, addition of δ-gluconolactone to the reaction mixture caused rapid decrease in the absorption at 340 mμ. (Fig. IV)

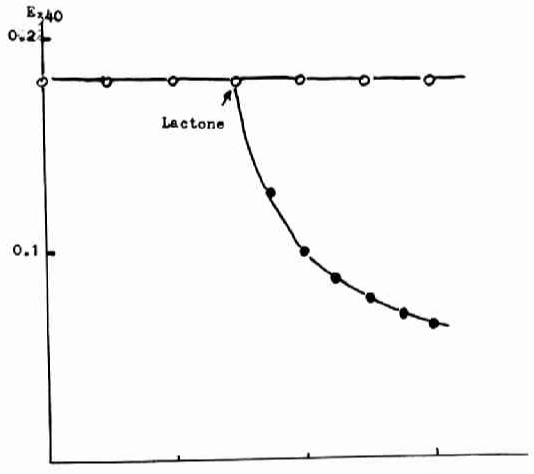

Fig. 4 Reversibility of the reaction
Assay conditions: see the text.

After the reaction was allowed to proceed in the presence of hydroxylamine, a reddish purple color developed when $FeCl_3$ was added. These facts suggested that the primary product of this reaction was not GA but gluconolactone and subsequent hydrolysis yielded GA just as in the cases of glucose dehydrogenases from other sources.

D I S C U S S I O N

Glucose dehydrogenase from the particulate fraction of

Acetobacter suboxydans has been shown by King and Cheldelin (1) to have an optimum pH between 5.0 and 5.5 and not to require any pyridine nucleotide as a hydrogen accepter even with the solubilized, purified preparation. Similar results were obtained by the author with the same organism used in this paper. On the other hand, the enzyme from the soluble fraction has now been shown to have several different characters: the optimum pH lies in the more alkaline range than that of the particulate enzyme and MADP is exclusively required as a hydrogen accepter. It is, therefore, suggested that the dehydrogenase from the soluble fraction has quite a different role from that of the particulate fraction in the oxidation of glucose. This enzyme seems, furthermore, to be different from the NAD specific one reported by King and Cheldelin (9) in another strain of Acetobacter suboxydans.

The enzyme was found to catalyze oxidation of mannose as well as glucose. Moreover, the Michaelis constant for mannose is smaller than that for glucose. Fairy constant ratio of the activity of the enzyme for glucose to that for mannose throughout the purification procedure suggests that oxidation of two aldoses is catalyzed by a single enzyme rather than by multienzymes. Acetobacter suboxydans was unable to grow in the medium containing mannose in place of glucose. When this microorganism grown on glucose was aerobically incubated with mannose, only one atom of oxygen was consumed per molecule of mannose and further oxygen uptake was not observed. These observations suggest that mannose is not utilized under the ordinary conditions.

S U M M A R Y

The primary step for the formation of 5KGA was studied with

the soluble fraction of Acetobacter suboxydans. The enzyme, glucose dehydrogenase, was separated from 5KGA reductase and was shown to be exclusively NADP linked. The optimum pH was about 8.5. Of the several substrates examined, only glucose and mannose were dehydrogenated by this enzyme and were proved to give corresponding aldonic acids stoichoimetrically. Some properties of the enzyme were compared with that of particulate enzyme.

The author wishes to express his grateful thanks to Professor Shozo Tanaka for his interest and encouragement.

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ENZYMATIC STUDIES ON THE FORMATION OF 5-KETOGLUCONIC ACID BY ACETOBACTER SUBOXYDANS

(II) 5-KETOGLUCONATE REDUCTASE

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Glucose is dehydrogenated in two ways to give GA[§§] by Acetobacter suboxydans: one is catalyzed by the enzyme in the particulate fraction, the other is by that in the soluble fraction. Characterization of the latter enzyme is described in the preceding paper (1). In the present paper, enzymatic studies on the subsequent oxidation of GA to give 5KGA by this microorganism will be described. Some physiological situation of this enzyme will be also discussed.

EXPERIMENTAL

Microorganism Acetobacter suboxydans IFO 3432 was used throughout this work. All the methods of cultivation and preparation of crude extracts and soluble fraction were the same as that described previously (1).

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§§ Abbreviations: GA, gluconic acid; 5KGA and 2KGA, 5 keto- and 2 keto-gluconic acid, respectively; Tris, tris-hydroxymethyl aminomethane.

Chemicals D-Mannoic, D-galactonic, D-xylonic and L-arabonic acids were prepared by the oxidation of corresponding sugar with bromine water. D-Lyxose was prepared by Ruff's degradation of D-galactonic acid. 5KGA was isolated as calcium salt from the culture medium of Acetobacter suboxydans, recrystallized from hot water, and used after conversion to sodium salt by treating with sodium oxalate or with Amberlite IR50 (Na^+ form). Calcium salts of L-idonic acid and 2KGA were kindly furnished by Dr. Yoshio Nozaki of Shionogi Pharmaceutical Co.

Analytical Methods 5KGA was determined spectrophotometrically by carbazole method reported by Suda et al. (2) with minor modification as follows: 0.5 ml. of sample was throughly mixed with 2.5 ml. of diluted H_2SO_4 (H_2SO_4 : H_2O = 6 : 1) in the cold. To this 0.1 ml. of alcoholic solution of carbazole (0.1%) was added. After incubation at 30°C for 30 minutes, absorbance at 540 mμ was measured. 2KGA was determined by the method of Lanning and Cohn (3). Ascending paper chromatography was performed with two solvent systems each in case of aldonic acid and keto aldonic acid. Rf values were summarized in Tab. I.

Table I

Paperchromatography of Aldonic Acids and Ketoaldonic Acids

Solvent	5KGA	2KGA
BuOH* : AcOH : H_2O (4:1:1)	0.60	0.43
BuOH : EtOH : H_2O (4:1:1)	0.52	0.40
Color	brown	pink

Color development : Aniline Phthalate (4)

Solvent	GA	IA	MA
BuOH : AcOH : H_2O (4:1:1)	0.50	0.44	0.38
BuOH : EtOH : H_2O (4:1:1)	0.51	0.45	0.34

Color development : NH_2OH - $FeCl_3$ (5)

Samples were applied after being converted into lactone, see in the text.

* Abbreviations : BuOH, Butanol; EtOH, Ethanol; AcOH, Acetic
IA, Idonic Acid; MA, Mannoic Acid.

The other methods of estimation were described previously (1).

Enzyme Assay Dehydrogenation of GA 50 μmoles of GA, 0.15 μmole of TPN and 0.1 ml. of the enzyme were incubated in the final volume of 3.0 ml. (Tris or $NaHCO_3$ buffer, 0.1 M, pH 9). The increase of the absorbance at 340 mμ was measured by Beckmen Spectrophotometer model DU.

Reduction of 5KGA The decrease of the absorbance at 340 mμ was measured in the following incubation mixture: 10 μmoles of 5KGA, 0.1 μmole of TPNH, 0.1 ml. of the enzyme in the final volume of 3.0 ml. (Tris buffer, 0.1 M, pH 8).

In each case, one unit is defined as the amount of the enzyme which causes 0.001 of the change at 340 mμ in one minute at 30°C.

RESULTS

Presence of 5KGA Reductase in the Soluble Fraction When GA was incubated with crude extract in the presence of DPN, TPN and the regenerating system of their reduced form, 5KGA as well as 2KGA were detected in the reaction mixture by paper chromatography. The same result was obtained with soluble fraction, while when washed particle fraction was used only 2KGA was produced. These fact suggested, essentially in good agreement with DeLey's observation (6), that 5KGA reductase is present only in the soluble fraction.

Partial Purification of the Enzyme All the procedures were carried out at 0-5°C unless otherwise specified.

Acrinol Treatment To 50 ml. of the soluble fraction (1) 5 ml. of 1% acrinol solution were added slowly with constant stirring. After the removal of yellow voluminous precipitate by

centrifugation, 4 ml. of 10% charcoal suspension were added. After standing for 10 minutes in the room temperature, charcoal was filtered off.

$(NH_4)_2SO_4$ Fractionation Solid $(NH_4)_2SO_4$ (14 g.) was added to the acrinol treated supernatant (40 ml.) to give a concentration of 55% saturation. The precipitate was centrifuged off and additional 4.6 g. of the salt were added (70% saturation). The resulting precipitate was dissolved in 6.5 ml. of Tris buffer. Typical example of the purification was shown in Tab. II.

Table II

Purification of the Enzyme

Step	Volume (ml.)	Total activity (unit)	Specific activity (units/mg.prot.)	Yield (%)
Soluble fraction*	50	33,200	74	100
Acrinol fraction*	59	21,300	147	64
$(NH_4)_2SO_4$ fraction (55 - 70%)*	10	9,180	568	28

* All the values were corrected for the amount used for pilot test during purification steps.

Properties of the Enzyme: Co-enzyme and Substrate Specificity Mannoic and idonic acids were found to be dehydrogenated in addition to gluconic acid although they are less active. Other aldonic acids tested were virtually inactive. (Tab. III)

Table III

Substrate Specificity

Substrate	per cent activity
D-Gluconic Acid	1 00
D-Mannoic "	28
L-Idonic "	12
D-Galactonic "	2
D-Xylonic "	0
L-Arabinic "	0
D-Glucuronic "	0

TPN was three times effective as DPN.

In the reverse reaction, TPNH was oxidized in the presence of 5KGA. 2KGA was not, however, reduced at a detectable rate although it is fairly rapidly reduced by TPNH in the crude extracts.

Relation between Equilibrium Constant and pH As was shown in Fig. I, entirely different optimum pH's were proved in the normal and reverse reactions. The addition of a large amount of GA was found to be necessary in order to push the following reaction to the right hand side.

$$GA + NADP \longrightarrow 5KGA + NADPH + H^{+} \quad \text{.....(I)}$$

Since the equilibrium constant (K) defined as (II)

$$K = \frac{(5KGA)(NADPH_2)}{(GA)(NADP)} \text{ (const. pH)} \quad \text{.....(II)}$$

was expected from equation (I) to be greatly influenced by pH,

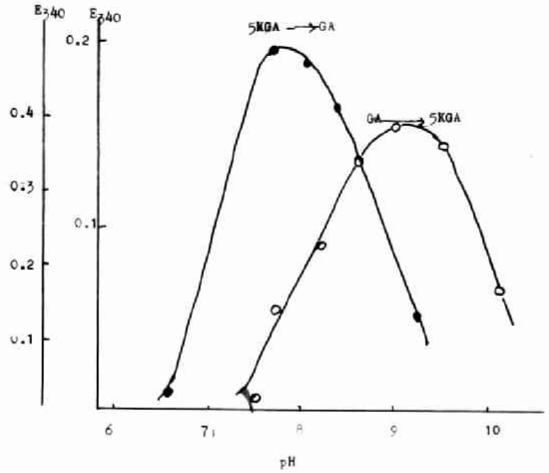

Fig. 1 Effect of pH on the enzyme activity
Assay conditions : see the text

several K values were determined at the several different pH's. Plotting of pK against pH shows that pK increased almost linearly as pH decreased. (Fig. II)

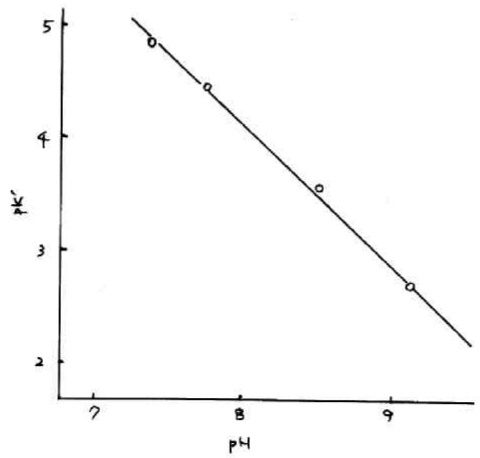

Fig. 2 Relation betweenn pH and pK^1

Assay conditions : see the text

Thus the K_H value including (H^+) was determined as follow:

$$K_H = \frac{(5KGA)(NADPH)(H^+)}{(GA)(NADP)} = 3.5 \times 10^{-12}$$

Reaction Products The product of the enzymatic dehydrogenation of GA was examined after relatively large scale incubation with the system in which TPN was regenerated. Incubation system contained 150 μmoles of GA, 300 μmoles of $(NH_4)_2SO_4$, 0.4 μmole of TPN, 3 mmoles of Tris, 9000 units of crystalline glutamic dehydrogenase§ (from beef liver) and 5 ml. of 5KGA reductase in the final volume of 30 ml. The pH of the solution was adjusted to 9.0 by 1 N H_2SO_4. After incubation for 3 hours at 30°C, the reaction was terminated by immersing the tube into the boiling water and the deprotenized solution (adjusted to pH 7.5) was treated with Amberlite IR120 (H^+ form). After $Ba(OH)_2$ was added to the solution to remove sulfate ion, the clear solution was concentrated in vacuo. The concentrated product was examined by two ways: Ascending paper chromatography in two solvent systems showed that the product is 5KGA. Any spot corresponding to 2KGA was not observed which is distinct from that of 5KGA in both Rf value and color. (Tab. I) The result was further confirmed by carbazole reaction having the absorption maximum at 540 mμ. (Fig. III) Under the condition described in EXPERIMENTAL, the reaction was found to be virtually negative for glucose, GA, 2KGA and glucuronic acid. 2KGA was not detected by o-phenylenediamine reaction (3).

Reduction of 5KGA is expected to yield two kinds of aldonic

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§ Kindly supplied by Dr. Watari of Osaka University.

acid, i.e., D-gluconic acid and L-idonic acid. Therefore the product of the reverse reaction was also examined.

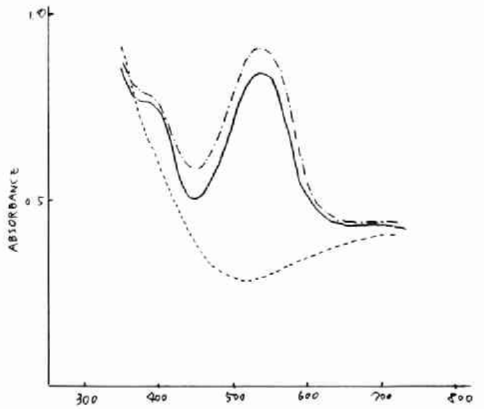

Fig. 3 Absorption spectra of reaction product and authentic samples.

Since the enzyme preparation was still contaminated with TPN-linked acetaldehyde dehydrogenase, incubation was performed with this TPNH generating system. The incubation mixture contained 0.5 μmole of TPN, 50 μmoles of 5KGA (K salt), 2 mmoles of Tris and 5 ml. of enzyme preparation in the final volume of 20 ml. The pH of the solution was adjusted to 7.5 with 1 N H_2SO_4. To the reaction mixture, 10 μmoles of acetaldehyde were added with stirring in several portions at 10 minutes intervals (1 ml. each). The reaction was allowed to proceed for 1 hour at 30°C. At the end of this period, after termination of the reaction by heating the sample, the solution was treated with Amberlite IR120 and $Ba(OH)_2$ as in the previous case. The resulting aldonic acid was

converted into lactone by heating it at 90°C for 3 minutes in 0.1 N HCl. Development of the sample by paper chromatography in two solvent systems (Tab. I) revealed one spot which is corresponding to gluconolactone. Therefore, majority of, if not all, the product of the reverse reaction is thought to be gluconic acid.

D I S C U S S I O N

Although the purification of the present preparation is not so achieved, most of 2KGA reductase is thought to be removed from the preparation because 2KGA was not detected in the reaction mixture and TPNH is not appreciably oxidized by 2KGA in the reverse reaction.

Equilibrium studies on 5KGA reductase revealed that the reaction is very unfavorable in respect to the formation of 5KGA under the physiological condition. Nevertheless, the bacterium produces a copious amount of 5KGA in the culture medium. The reason for this is at least in part explained by the fact that Ca salt of 5KGA is hardly soluble in water (2.6 mg./ml. as free acid) and the removal of 5KGA from the system pulls the reaction (I) to the right hand side. However, as it is obvious from the equation (I), considerably active TPNH oxidizing system should be conjugated to maintain the concentration of 5KGA at this level (Ca 10^{-2} M) under the physiological pH range ((H^+) is about 10^{-2} M). Of the several reactions examined involving TPNH oxidation, only glyoxylate oxidase was very active in crude extracts.§ The TPN-linked glyoxylate oxidase has recently been

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§ Okamoto: Unpublished experiment.

reported in the other bacterial extracts (7).

Since glucose dehydrogenase in the soluble fraction is also TPN-linked (1), relation among these enzymes participating in the formation of 5KGA and glyoxylate oxidase should be further examined for the elucidation of physiological significance of 5KGA formation, because it seems closely related with the oxidation-reduction systems of the bacterium.

S U M M A R Y

5KGA reductase which catalyzes the reaction GA + TPN + H_2O $\rightleftarrows$ 5KGA + TPNH + H^+ was partially purified from the soluble fraction of Acetobacter suboxydans. The enzyme was found to have an optimum pH of about 7.5 in the forward reaction (GA $\rightarrow$ 5KGA) and that of about 9.5 in the reverse reaction. TPN was three times as effective as DPN. Mannoic and idonic acids in addition to gluconic acid were shown to be reduced at a detectable rate. Examination of the reaction product proved that 5KGA and gluconic acid were produced in the forward and reverse reactions, respectively. The equilibrium studies revealed that this reaction was very unfavorable in respect to the formation of 5KGA. (K_H = 3.5×10^{-2}) Some phydiological situation of the enzyme in the formation of 5KGA was discussed.

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